

Influence of Predation Risk on Male Courtship and Mate Choice in Sailfin Mollies,
Poecilia latipinna

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ABSTRACT

Females are typically considered the “choosier” sex, however males may express a great deal choice under certain circumstances (sperm limitation and competition, parental investment, and resource limitation, etc.). Despite the strong effects of predation risk observed on female choice, few studies have examined to role of risk on shaping male mate choice. Herein we reviewed the literature on male mate choice and predation risk. We then presented the results of a study of the effects of varying levels of predation risk on the male mate choice in sailfin mollies (*Poecilia latipinna*).

The presence of a predator (at both high and low levels of predation risk) had no affect on the overall levels of male receptivity. Predation risk also did not affect the degree to which males preferred to associate with a conspecific female over a heterospecific, sexual parasite, the amazon molly (*Poecilia formosa*). In the absence of predation risk, however, males did show a significant preference for displaying to amazon mollies. This preference was extinguished at both levels of predation risk. These results indicate that, if anything, the presence of predation risk makes males less choosy about which female they choose to court.

BACKGROUND

Mate choice often involves the delicate balancing act of trying to maximize reproductive benefits while at the same time reducing reproductive costs. For organisms that reproduce asexually, mate choice is hardly a concern, but for the large majority of animals that reproduce sexually, reproduction doesn't occur without a partner. Since an organism's fitness is measured in the number of viable offspring they can produce, these pairings can be vitally important.

Selecting the right mate can lead to a whole host of benefits such as obtaining a mate with better fertilization ability or fecundity, receiving higher parental investment, and producing offspring with higher heritable viability or other qualities (e.g., Parker 1970, Rutowski 1982, Forsberg 1987, Oring and Lank 1986, Oring *et al.* 1991a,b, Williams 1975, Partridge 1980, Taylor *et al.* 1987). Not only do individuals stand to produce more viable offspring, they can also receive more immediate, tangible benefits such as being provided with more food or other resources, obtaining superior breeding territories, lowering their risk of predation, harassment or other hazards and reducing their risk of contracting parasites or sexually transmitted diseases (e.g. Thornhill 1976, Gwynne and Simmons 1990, Campanella and Wolf 1974, Trail and Adams 1989, Borgia and Collis 1989, Crowley *et al.* 1991).

While the benefits of mate choice can be lucrative, the costs of selecting an inferior mate can be equally detrimental. Individuals stand to lose precious time, energy and resources that can eat away at their reproductive fitness as well as their own survival rates (Alcock 2005). While these and other costs apply to both genders, females tend to carry the brunt of these expenses and are therefore commonly considered the "choosier"

sex. One of the greatest reasons for this disparity between the genders is the asymmetry in initial investment. Females produce few, energetically expensive eggs while males produce many, cheap sperm. While some have suggested that the sum total of energetic investments may be equal, empirical testing has revealed an even bigger asymmetry in energetic investment than predicted (i.e. Ryan *et al.* 1983). With such a huge difference in energy expenditures for initial investment, females stand to lose far more from failed or low quality matings than males do.

Also, while males tend to increase their number of offspring with multiple matings, females do not. For example, male *Drosophila* had a directly proportional increase in the number of young sired as compared to the number of females they inseminated. Females, however, had no marked increase in the number of offspring they produced when they mated with multiple males (Bateman 1948). Therefore, while it often behooves males to try to copulate with multiple females, females often do not increase their reproductive fitness by mating with larger numbers of males.

Male Mate Choice

Despite the general pattern of strong female choice, males can exhibit preferences as well, particularly in species where females have a marked difference in fecundity (e.g., Parker 1970, Rutowski 1982, Forsberg 1987). For example, when fecundity is related to body weight or size it makes more sense for males to choose the largest and thus most fecund females. This is especially true if the male must balance costs such as time, energy, depleting sperm or other resources, and reducing their chances of fertilizing other females (Andersson 1994).

Another important factor for males to consider when selecting a mate is sperm competition (Parker 1970). Once a female has already mated, particularly for a certain period of time, the chance of successful fertilization by a second male goes down significantly. Therefore males tend to avoid copulation with females that have already mated, especially within a certain time frame.

Due to sperm limitation, males may even refuse to do repeated copulations with the same female. For example, in lekking species where females competed for repeated copulations, males often refuse to inseminate the same female more than once (Seaterh 2001). Sperm limitation has actually been credited as being one of the biggest contributors to male mate choice since sperm quantity has been directly linked to fertilization success (Pennington 1985, Levitan *et al.* 1992, Yund and McCartney 1994). Just as females benefit from choosing males with increased sperm abundance, males also stand to benefit by choosing females with larger eggs. That way males are ensured a greater chance of reproductive success even if their own sperm quantity is a limiting factor (Levitan 1998).

In many species, males present females with different nuptial or nutritional gifts as part of the courting behavior. The added nutrition tends to make females more fecund and can therefore actually make males a limiting resource for which females compete (Thornhill 1976). Gwynne and Simmons have shown that certain female katydids that receive nuptial gifts of a spermatophore from males actually compete sexually for mates when other food sources are scarce (Gwynne and Simmons 1990). Further, female long-tailed dance flies have been shown to exploit male mate choice by sending an unreliable

message that deceives males into thinking their eggs are nearing maturation so they can obtain a protein meal in exchange for copulation (Funk *et al.* 1999).

Just as with nuptial gifts, parental care can be another factor that makes males into a limiting resource for which females compete. In several species where males are the primary or only caregiver, females have undergone sex role reversals by courting and/or competing over mates and breeding territories (Oring and Lank 1986, Oring *et al.* 1991a,b, Williams 1975). In these cases it is the male that largely decides if the mating will take place and sometimes actually declines female invitations (Smith 1979a,b, 1980, Kruse 1990).

Based on nuptial gifts and parental care, one might also assume that mating cannibalism (where the female eats her mate after copulation) would be another arena for male mate choice. Since the male is the ultimate limiting resource, sacrificing himself to increase female fecundity, males may be much more selective about which females they chose to court. In empirical studies, however, it appears that the males are far from willing to be cannibalized so easily and they tend to approach females cautiously and move away quickly after copulation (e.g. Liske and Davis 1987, Birkhead *et al.* 1988, Polis and Sisson 1990, Elgar 1992). Male choice may still exist but in a slightly different fashion. For example, males are more willing to court larger females only after they have already captured and consumed alternative prey (Robinson and Robinson 1980, Elgar *et al.* 1990). Similarly, orb-weaving spiders commonly attempt to mate with females only after they have just finished molting so they are incapable of attack (Robinson and Robinson 1980).

Predation Risk affects Mate Choice

Although many experiments have looked at causes of male mate choice (such as sperm limitation and competition, parental investment, and limited resources), one potential contributor generally overlooked: predation risk. Many of these mate choice experiments occur in artificial laboratory settings where the actors are removed from any kind of predation risk. In wild conditions, however, predation is a common and significant factor that impacts animal behavior.

For example, both males and females have been shown to reduce their mating search time and number of courtship displays when their perceived risk of predation was higher (e.g. deRivera *et al.* 2002, Koga *et al.* 1998, Su and Li 2006, Taylor *et al.* 2005). This reduction in courtship and insemination time makes sense considering that choosier males have higher mortality rates (Kasumovic *et al.* 2006).

Interestingly though, a reduction in mate sampling is not always tied to a reduction in courtship or copulations. In many taxa, females maintain consistent levels of both behaviors while under increased predation risk, the only difference being that they are more willing to mate with closer males (Dunn and Whittingham 2007, Karino *et al.* 2000). Therefore, it is the reduction in mate sampling that makes females less choosy, not the courtship or mating behavior itself.

Behavioral modifications due to predation risk can also impact several species at once. For example, with mixed-species frog choruses, when members of the lead species stop calling due to a perceived predation risk, the heterospecific eavesdroppers follow suit to avoid predation as well (Tuttle *et al.* 1982, Phelps *et al.* 2007).

These examples suggest that predation risk does have an impact on male mating behavior, but how significant this relationship is remains to be seen. Further, the

question arises that as the level of predation risk changes, do males modify their mating behavior accordingly? Finding the answers to these questions might give us increasing insight into yet another complex layer of male mate choice.

Study System

In order to study the effects of predation risk on male mate choice, we used sailfin mollies (*Poecilia latipinna*) and the closely related species, amazon mollies, (*Poecilia formosa*). Sailfin and amazon mollies have an interesting evolutionary relationship. The amazon molly is a unisexual gynogenetic species that requires the sperm from males of closely related bisexual species to initiate embryogenesis (Turner 1982 & Dawley 1989). In essence, this means that the amazon mollies are an all female species that sexually parasitize males of the parental species (*P. latipinna* and *Poecilia mexicana*) to produce their clonal offspring (Turner 1982 & Dawley 1989).

Male sailfin mollies that mate with the parasitic amazons gain no offspring that contain their genetic information and therefore do not increase their fitness, which begs the question as to why males would take part in such an evolutionary disadvantageous process (Aspbury 2004). Although the answer to this puzzling question is still unresolved, one possible explanation is that males stand to benefit from mate-choice copying. Mate-choice copying is when one female chooses to mate with a particular male after observing him mate with another female (Pruett-Jones 1992). It has been shown that female sailfin mollies copy the mate choice of the heterospecific amazon mollies. Therefore, male sailfin mollies may stand to increase their future reproductive success (indirectly) by mating with the amazon mollies (Schlupp *et al.* 1994).

The objective of this study is to test the effects of predation risk on male mate choice. It is predicted that the increased costs associated with the presence of a predator, the large mouth bass (*Micropterus salmoides*), will cause male sailfin mollies to be choosier about which females they court, a heterospecific (amazon molly) female or a conspecific female. We predict that males will be the least choosy when there is no predation risk. As predation risk increases, however, so too should male choosiness. Results from this study will be instrumental in examining new factors that could potentially impact male choosiness and mating behavior in addition to giving insight into how predation risk affects mate choice as a whole.

METHODS

Sailfin mollies and amazon mollies were collected by seining from Comal Springs in New Braunfels, TX. Largemouth bass were acquired from private pond stocking suppliers at Lochow Ranch in Milano, TX. Fish were maintained in glass aquaria in the lab and fed daily. The care and testing of all animals were performed in accordance with IACUC policies under IACUC Protocol #07082101.

Predation stimuli were constructed using video recordings of the bass. Video playback of predators has been used successfully in other Poeciliid fishes (Johnson *et al.* 2003). We divided a 90 cm x 32cm x 32cm aquarium into three, equal-sized sections along its length using three clear acrylic dividers (see Fig. 1). To film the "predator absent" stimulus, the tank was filmed with water but no bass. To film the "low predation" treatment, the bass was placed in the furthest part of the aquarium. The "high predation" treatment was filmed with the bass in the section closest to the camera. Each stimulus

was recorded for 6 minutes. Using the same video recordings in all tests, rather than the live bass, avoided any influences of variation in the predator's behavior.

The testing chamber consisted of another 90 cm x 32 cm x 32 cm aquarium. We divided this tank into three sections along its length (a central 60 cm section and two 15 cm sections at the ends) using clear, perforated, acrylic dividers (see Figure 1). The sides and back of the tank were covered in black plastic. A window was cut into the back plastic covering the central section of the tank to allow an LCD monitor to be projected into the central section of the aquarium. The LCD monitor was positioned at a distance that would allow only the animal in the central chamber to see the screen, which was verified using video recordings taken from inside the side chambers.

Males were selected randomly and added to the central chamber of the aquarium. Each male was assigned a random order of the three predation risk treatments as well as a random location for the amazon molly (either left or right) using a number generator. A female sailfin molly and a female amazon molly were added to their assigned chambers. The fish were given two minutes to acclimate before the predation risk treatments were played in their assigned orders for 5 minutes each. The behavior of the male was recorded for the fifteen minutes the predation treatments were being presented. The camera was positioned so that only the male was in view.

The recordings were later played back and analyzed using Jwatcher (www.jwatcher.ucla.edu). We recorded the location of the male at all times during the recordings, also making note of the occurrences of visual displays (fin spreading). The observer was blind to the identity of the female on each side, but could, unavoidably, see the predation risk treatment.

Male preference was assessed independently using the association time data and display rates. The data were analyzed using a repeated measures ANOVA design to control for individual differences in overall level of male behavior.

RESULTS

Predation risk had no significant effects on either overall association rates with females ($F = 0.328$, $df = 18$, $p = 0.144$), or total display rates ($F = 1.609$, $df = 18$, $p = 0.231$). Under repeated measures ANOVAs, the level of predation risk had no effect on the degree to which males preferred to associate with the conspecific females ($F=0.377$, $df=16$, $p=0.692$). In the absence of predation risk, males were significantly more likely to display to the amazon molly ($t=-2.27$, $df=17$, $p= 0.018$). The low and high predation risk, the males do not show a preference.

The sizes of Amazon mollies and females used in the study were not significantly different ($t= 1.53$, $df=18$, $p=0.144$), nor were these sizes significant covariates in any statistical tests on male behavior. Male size was not a significant covariate in any measure of male behavior, though larger males tended to have higher overall display rates than smaller males.

DISCUSSION

Unlike previous sailfin and amazon mate choice experiments (where male sailfins had a preference for conspecifics), males had no significant preference for sailfins in any of the treatment levels (see Fig. 4). The presence of a predator (at both high and low levels of predation risk) had no affect on overall levels of male receptivity (see Fig. 2). Predation risk also did not affect the degree to which males preferred to associate with the conspecific female nor the rate at which they made displays (see Fig. 3 and Fig. 4).

In the absence of predation risk, however, males did show a significant preference for displaying to amazon mollies (see Fig. 5). This preference was extinguished at both levels of predation risk. These results indicate that, if anything, the presence of predation risk makes males less choosy about which female they choose to court. Although this is contrary to what we proposed, this pattern is not wholly surprising considering that females from many taxa have been shown to decrease choosiness when perceived predation risks are high (e.g., Dunn *et al.* 2007, Dunn and Whittingham 2007, deRivera *et al.* 2002, Karino *et al.* 2000).

One explanation for this phenomenon in females is that unless indirect benefits are large, predation makes the energetic costs of mate sampling is too substantial (Byers *et al.* 2005). Applying this idea to this study, would mean that the indirect benefits male sailfins receive by courting amazon mollies (through mate-copying) is not enough to offset the costs of predation risk. This may actually mean that males are most vulnerable when traveling between females instead of when courting them. Therefore, males may still retain high association times and display rates when predators are present but instead reduce travel time and mate sampling.

One potential problem with this study was that the video playback. Although video playback has been shown to be successful with other Poeciliid fishes (Johnson *et al.* 2003), it may have been insufficient for our work with sailfin mollies. One change that could be made is using a live predator in each of the trials. Also, the playback system did not allow the males to detect any olfactory cues that might have been excreted from the predator. Although these olfactory cues might have impacted male behavior,

allowing for their interchange would have been problematic. In order for females to remain unaware of the predator's presence female cues would have to be removed.

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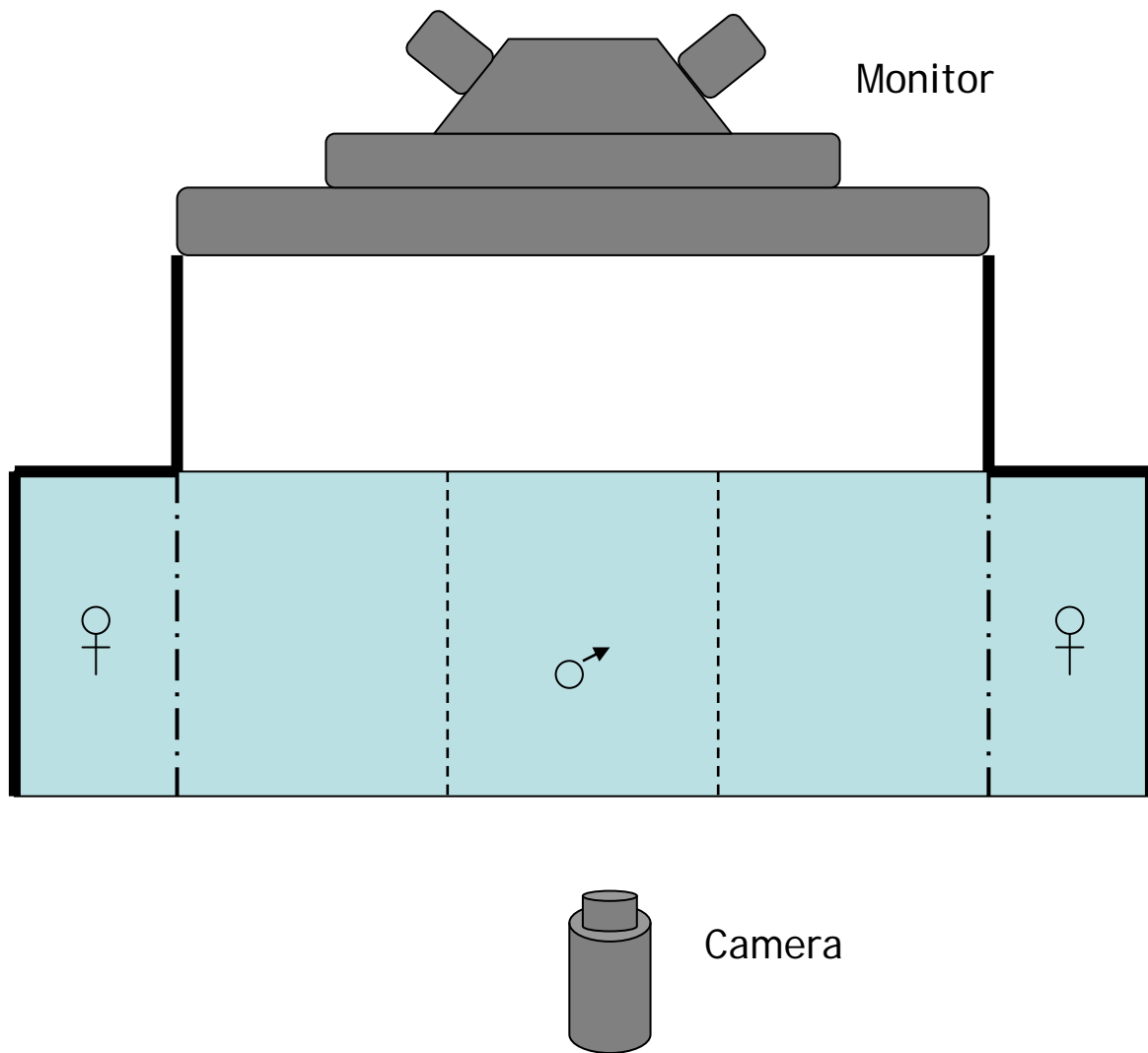


Figure 1: Diagram of experimental setup as seen from above. The angle between the female compartments and the monitor was designed to prevent females from seeing the monitor. Visual obstruction was verified by video recorded inside the compartment.

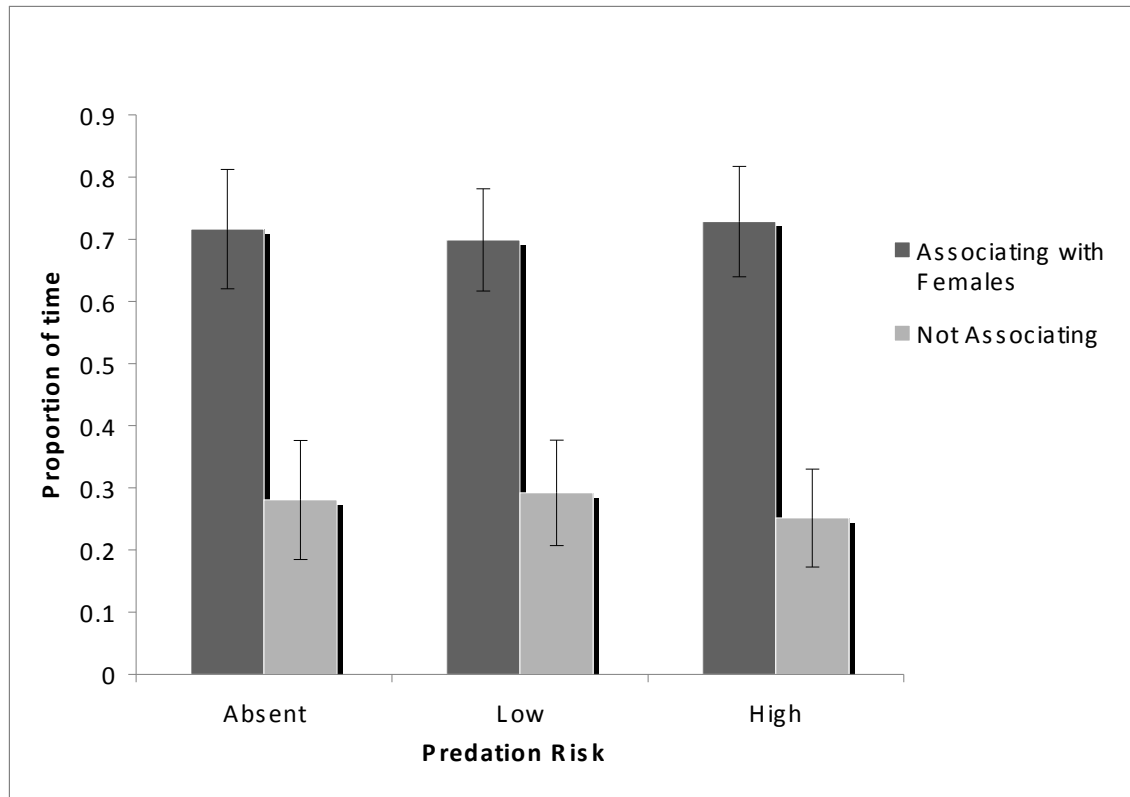


Figure 2. Association time with females for a male sailfin molly, *Poecilia latipinna*, under three levels of predation risk simulated using video playback of a predatory bass. In each of the three treatment levels, males did not show any change in their preference to associate with females.

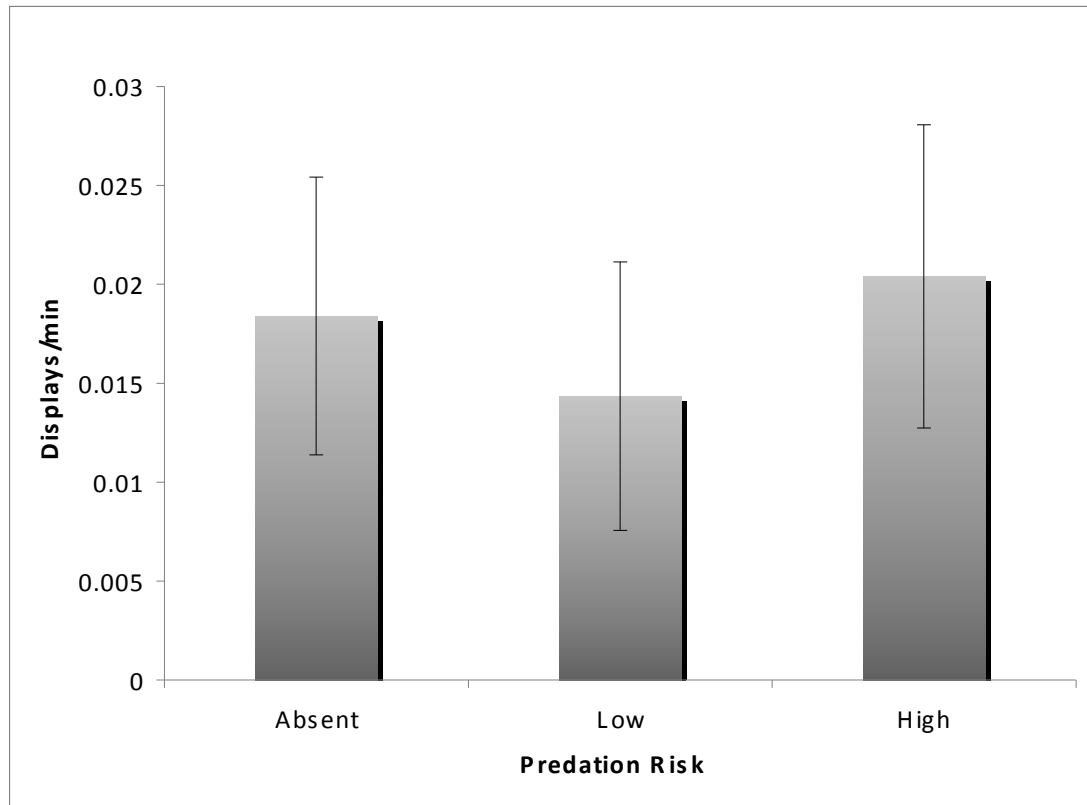


Figure 3. Overall rates of male courtship displays to females under three levels of predation risk simulated using video playback. In all three treatments males showed no significant change in their display rate.

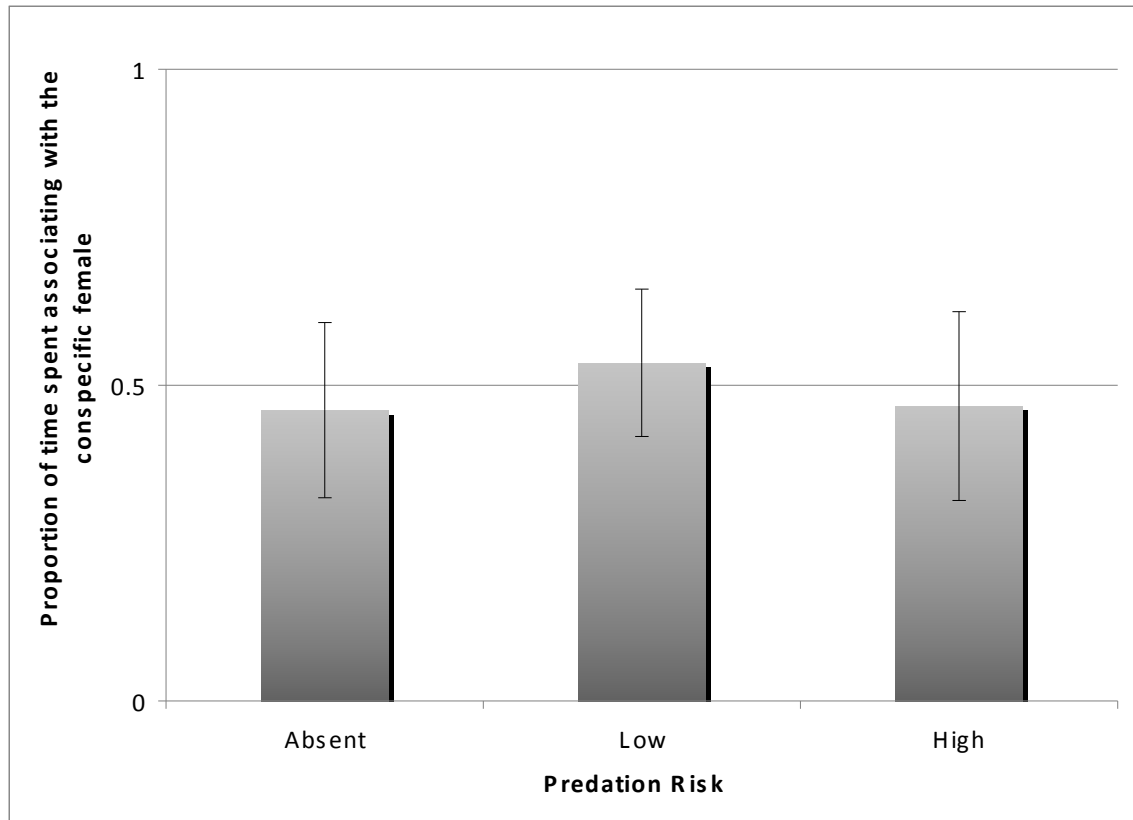


Figure 4. Male preference for associating with the conspecific female over the heterospecific sexual parasite under three levels of predation risk, simulated using video playback. The line at 50% indicates no preference. In all three treatments males showed no preference for the conspecific female over the heterospecific female.

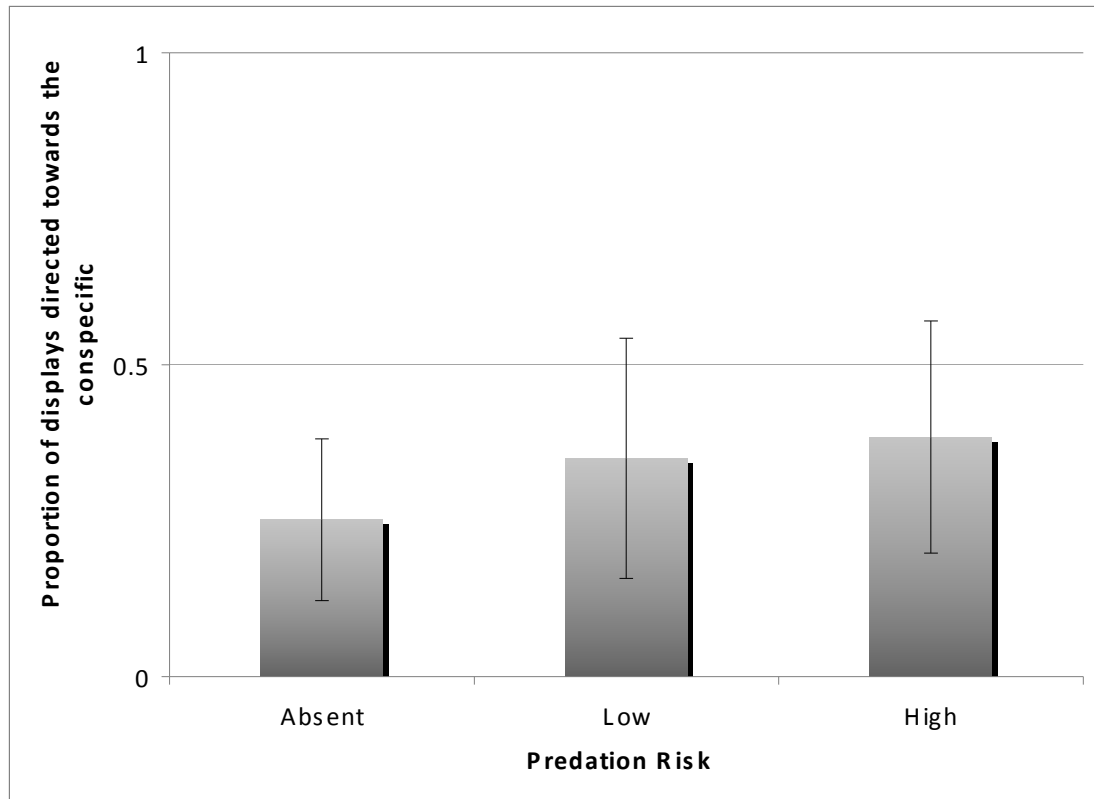


Figure 5. Male preference for displaying to the conspecific female over the heterospecific sexual parasite under three levels of predation risk, simulated using video playback. The line at 50% indicates no preference. In the treatment where predation risk was absent, males showed a significant preference for displaying to the heterospecific female. In both the low and high predation risk treatments, however, this pattern was lost and males showed no significant preference for displaying to conspecifics over heterospecific females.